J. Parasitol., 88(5), 2002, pp. 1029–1032

Disseminated Toxoplasmosis in a Mediterranean Pregnant Risso's Dolphin (*Grampus griseus*) with Transplacental Fetal Infection

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ABSTRACT: Fatal disseminated toxoplasmosis was diagnosed in a Risso's dolphin (*Grampus griseus*) dam and its fetus on the basis of pathologic findings, immunohistochemistry, and structure of the parasite. The dolphin was stranded alive on the Spanish Mediterranean coast and died a few hours later. At necropsy the dam was in good condition. From the standpoint of pathology, however, it had generalized lymphadenomegaly and splenomegaly, enlargement of and multifocal hemorrhage in the adrenal glands, diffuse mucosal hemorrhage of the glandular and pyloric stomach, ulcerative glossitis and stomatitis, focal erosions and reddening of the laryngeal appendix, and severe paraotic sinusitis with intralesional nematodes *Crassicauda grampicola*. The dolphin was pregnant, most probably in the first gestational trimester. The most prominent microscopic lesions were multifocal granulomatous encephalomyelitis, diffuse subacute interstitial pneumonia, mild multifocal necrotizing hepatitis and nonsuppurative cholangiohepatitis, gastritis and adrenalitis, mild lymphoid depletion, medullary sinus and follicular histyocitosis, and systemic hemosiderosis. The fetus had foci of coagulative and lytic necrosis in the kidneys, the lung, and the heart. Most lesions were associated with tachyzoites and tissue cysts of *Toxoplasma gondii*. The diagnosis was confirmed immunohistochemically. This is the first report on toxoplasmosis in a Risso's dolphin (*G. griseus*) and on transplacental transmission to an early-stage fetus in any cetaceans.

Toxoplasma gondii is of worldwide distribution in all warm-blooded mammals, including marine mammals (Dubey and Beattie, 1988). Wild and domestic felids are the only known definitive hosts, shedding oocysts in feces. *Toxoplasma gondii* can be transmitted to all hosts by ingestion of food and water contaminated with oocysts, by ingestion of infected meat, or congenitally, by transplacental infection with tachyzoites when infection is acquired during pregnancy (Dubey and Beattie, 1988). Parasitemia during pregnancy can cause the spread of *T. gondii* to the fetus. In humans, sheep, goats, and pigs, transplacental infection occurs mainly if the dam becomes infected for the first time during pregnancy, and the severity of disease in the fetus depends on the stage of pregnancy (Dubey and Beattie, 1988). Fatal toxoplasmosis has been previously reported in a wide variety of captive and wild marine mammals other than cetaceans (Holshuh et al., 1985; Garell, 1979; Lindsay et al., 2001). A few cases of toxoplasmosis have been documented in cetaceans, including a *Sotalia guianensis* (Bandoli and Oliveira, 1977), a spinner dolphin (*Stenella longirostris*) (Migaki et al., 1990), 2 Atlantic bottlenose dolphins (*Tursiops truncatus*) (Cruickshank et al., 1990; Inskeep et al., 1990), 4 stripped dolphins (*Stenella coeruleoalba*) (Domingo et al., 1992), 2 beluga whales (*Delphinapterus leucas*) (Mikaelian et al., 2000), and 1 Indo-Pacific bottlenose dolphin (*T. aduncus*) (Jardine and Dubey, 2002). A serological survey of 22 St. Lawrence Estuary beluga whales indicated an overall seroprevalence of 27% (Mikaelian et al., 2000), suggesting that *T. gondii* is widespread in this pelagic population.

An adult, 292-cm-long female Risso's dolphin (*Grampus griseus*), was found stranded alive on the Spanish Mediterranean coast in July 1999. It died a few hours after being transported to the local marine animal rehabilitation center. Samples of lung, heart, liver, spleen, pancreas, adrenal gland, kidney, intestine, stomach, tongue, brain, skin, larynx, paraotic sinus, lymph nodes, tonsil, uterus, ovary, and the entire fetus were fixed in neutral buffered 10% formalin, embedded in paraffin, cut at 5 μm, and stained with hematoxylin and eosin. Periodic acid Schiff (PAS) staining was performed on selected tissues. Immunohistochemistry was performed to screen for reactivity to markers of *T. gondii* in brain, liver, lung, lymph nodes, spleen, heart, pancreas, adrenal

gland, kidney, tonsil, stomach, intestine, uterus, and fetal tissues using a rabbit polyclonal antibody, as previously described (Lindsay and Dubey, 1989). Similarly, the brain and the lymph node were tested for phocine distemper virus (PDV) using a monoclonal antibody against the F protein of PDV (Trudgett et al., 1991), as previously described (Domingo et al., 1992). This antibody cross-reacts with dolphin morbillivirus (DMV) and porpoise morbillivirus. The selected sections of the brain, the kidney, and the liver of the dam and all fetal tissues were reacted with bradyzoite-specific anti–BAG1 *T. gondii* rabbit antibody, as described by McAllister et al. (1996).

At necropsy the body of the dam was in good condition. The most relevant gross lesions were generalized lymphadenomegaly, especially in the cervical and mediastinal lymph nodes, and splenomegaly. The adrenal glands were congested and enlarged and had multiple petechiae on the cut section. The uterus contained a 12.5-cm long, nonautolyzed fetus and an ovary with a prominent luteal body. There was also diffuse mucosal hemorrhage of the glandular and pyloric stomach. Apart from sand, water, and a few cephalopod beaks, we did not find any ingesta in the first stomach compartment. Sand was also found in the esophagus and the secretory stomach. The intestinal content was scant and green-colored. Other findings included a reddish frothy fluid in the caudal portion of the trachea, hydropericardium, multiple ulcers, up to 1 cm in diameter and covered with fibrinous exudate in the margins of the tongue and gingivae, focal mucosal erosions and reddening of the laryngeal appendix, severe paraotic sinusitis with multifocal mucosal mineralization, numerous nematodes (*Crassicauda grampicola*) attached to the mucosa, and 9 copepods (*Penella* spp.) attached to the flanks.

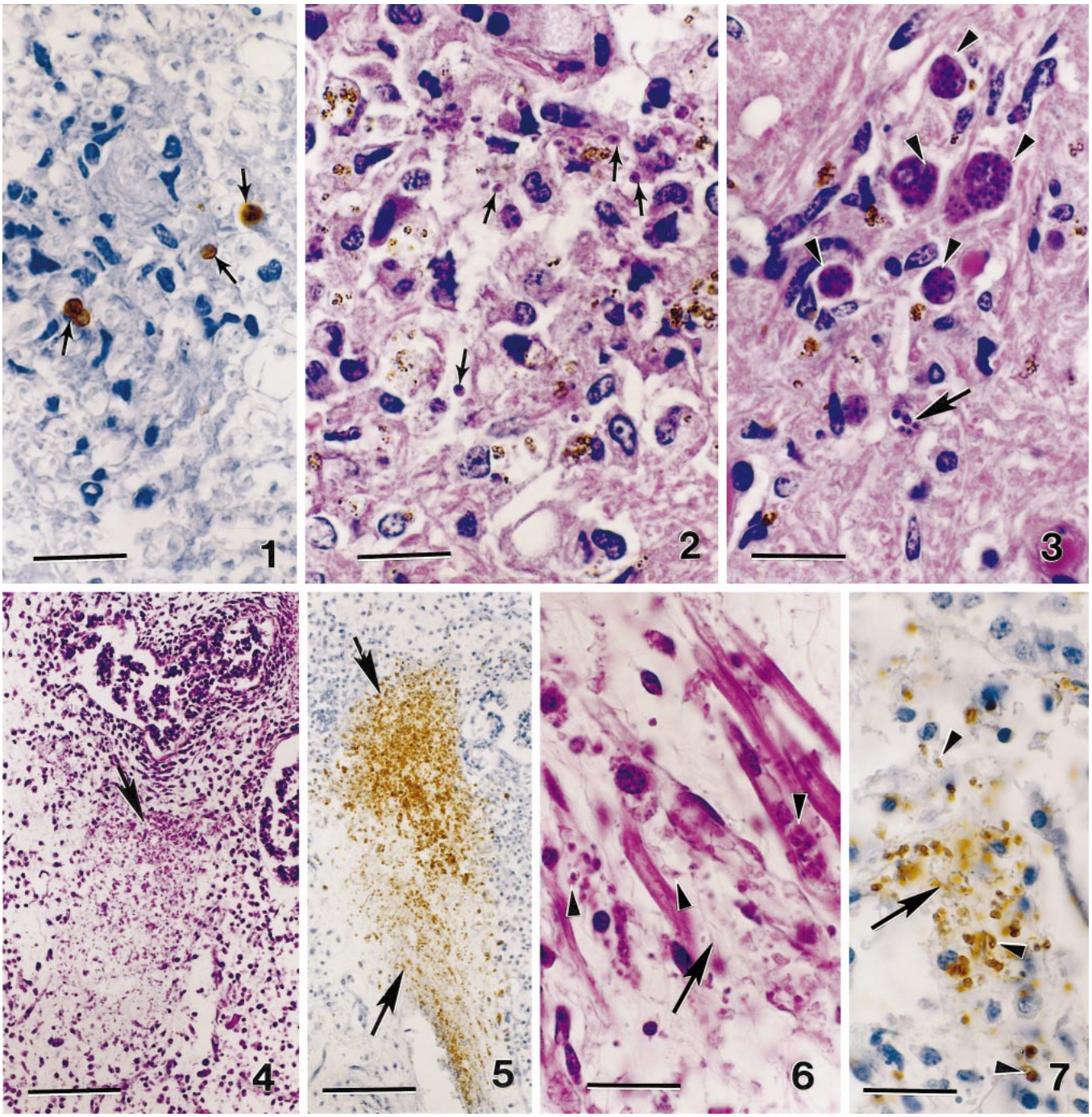
Histopathology of the dam revealed severe lesions affecting especially the brain, lung, adrenal glands, liver, stomach, and lymphoid tissues. There was a multifocal nonsuppurative inflammation in the central nervous system affecting the cerebrum, brainstem, cerebellum, and spinal cord, characterized by foci of gliosis and perivascular cuffing of mononuclear cells, mostly macrophages, in the white and gray matter (Figs. 1–3). Scattered perivascular edema and hemorrhages were also noted. Choroid plexi were multifocally infiltrated with mononuclear cells. Protozoan tissue cysts and extracellular or intracellular tachyzoites were often associated with these lesions (Figs. 1–3). Occasionally, clusters of tissue cysts were seen without surrounding inflammation. Tissue cysts were up to 25 μm in diameter; they included numerous PAS-positive bradyzoites surrounded by a thin cyst wall. Tachyzoites were round, PAS negative, and mostly found in variable size groups. The lung was diffusely affected by mild to moderate subacute interstitial pneumonia. The alveolar septa were thickened by infiltrates of the lymphocytes and the plasma cells, and the alveolar spaces were filled with some neutrophils and foamy macrophages, occasionally with intracytoplasmic groups of tachyzoites. Scattered throughout the adrenal cortex were multifocal foci of necrosis, with mild infiltration of lymphocytes and plasma cells. Intralesional groups and individual tachyzoites were seen either extracellularly or within the cytoplasm of adrenocortical cells. There was mild to moderate multifocal necrotizing hepatitis characterized by small foci of lytic necrosis associated with tachyzoites and infiltrates of mononuclear inflammatory cells. A few tissue cysts were identified in the cytoplasm of hepatocytes adjacent to necrotic areas. Additional hepatic lesions consisted of moderate nonsuppurative cholangiohepatitis, diffuse hepatic lipidosis, and severe hemosiderosis of hepatocytes and Kupffer cells. The subcapsular and medullary sinuses of lymph nodes were expanded by edema fluid and had numerous histiocytes containing hemosiderin. Moreover, in all lymphoid tissues, germinal centers were not observed, and there was mild follicular histiocytosis. In the tonsil, tachyzoites could be seen inside the follicular histiocytes. The secretory stomach had small foci of glandular epithelial necrosis, infiltrated with a few neutrophils and mononuclear cells, and tachyzoites within the cytoplasm of mucosa epithelial cells. Mild multifocal mononuclear enteritis was also associated with intracytoplasmic tachyzoites in enterocytes. Tissue cysts were sporadically present in cardiomyocytes without inflammation or necrosis. Histological lesions in the endometrium consisted of mild diffuse superficial infiltration of mononuclear cells; protozoans were not seen. Other findings consisted of superficial bacterial necrotizing and pyogranulomatous glossitis and laryngitis, with hemorrhages and vascular thrombosis, and mononuclear sinusitis. Microscopic lesions in the fetus were severe and observed in the kidneys, the heart, and the lung (Figs. 4–7). Scattered throughout the kidneys were intense focal areas of lytic necrosis associated with numerous extracellular or intracytoplasmic tachyzoites. In addition the lung and the heart also had moderate multifocal lytic or coagulative necrosis (or both).

The diagnosis was confirmed by positive staining of tissue cysts and tachyzoites with the anti–*T. gondii* polyclonal antibodies. *Toxoplasma gondii* was found infecting the macrophages, glial cells, endothelial cells, hepatocytes, cardiomyocytes, adrenocortical cells, gastric epithelial cells, intestinal crypt epithelial cells, and fibroblasts. Immunolabeling of organisms was most marked in the brain and liver of the dam and in the kidneys, lung, and heart of the fetus. In the fetus, large positive-stained areas were associated with necrotic foci. The numerous free tachyzoites, and groups of tachyzoites, observed in the glomerular mesangium, tubular epithelium, and interstitium of the kidneys were striking. Occasionally, small groups of tachyzoites were detected in the lung, fibromusculature, and neural fetal tissues. BAG1-positive bradyzoites were found in the brain and the liver of the dam but not in fetal tissues, suggesting that infection in the fetus was recently acquired. Immunohistochemistry for PDV was negative.

This is the first report of toxoplasmosis in a Risso's dolphin (*G. griseus*) and of vertical transmission to the fetus. Disseminated toxoplasmosis was the cause of death in the dam but apparently did not cause the premature fetal death. The pathologic findings and the localization of the protozoans were similar to other reports of *T. gondii* infection in cetaceans (Bandoli and Oliveira, 1977; Cruickshank et al., 1990; Inskeep et al., 1990; Migaki et al., 1990; Domingo et al., 1992; Mikaelian et al., 2000). After ingestion, bradyzoites from the tissue cysts or sporozoites from the oocysts penetrate the intestinal epithelial cells and multiply in the intestine. Then, *T. gondii* may spread first to mesenteric lymph nodes and then to distant organs by the invasion of lymphatics and blood where intracellular growth of tachyzoites causes necrosis (Dubey et al., 1997). The protozoans were found in almost all tissues, generally in close association with necrotizing and mainly nonsuppurative inflammatory lesions. In addition *T. gondii* was identified in the epithelial cells of the intestinal and gastric mucosa, suggesting an oral route of infection. No gross lesion was observed in the placenta. However, necrotic lesions associated with *T. gondii* were seen in the fetus and were similar to lesions described in transplacentally infected fetuses in other species (Dubey et al., 1990; Dubey and Beattie, 1988; Jardine and Dubey, 2002). In contrast, sheep and goats usually have gross placental lesions that consist of focal necrosis and mineralization of cotyledons (Dubey et al., 1990).

Whether generalized toxoplasmosis in the dam dolphin was caused by immunosuppression is unknown. Serologic data in beluga whales (Mikaelian et al., 2000) suggested the occurrence of latent infections in cetaceans, which argues against the high susceptibility of dolphins to *T. gondii* infection suggested by other authors (Migaki et al., 1990; Oksanen et al., 1998). Morbilliviruses are the only known immunosuppressive pathogens in cetaceans (Domingo et al., 1992; Lipscomb et al., 1996). Other potential factors include environmental contamination with polychlorinated biphenyls (PCBs) (Borrell et al., 1996). Toxoplasmosis has been found associated with morbillivirus infection in 4 striped dolphins (Domingo et al., 1992) and was probably a secondary infection to DMV in 3 other bottlenose dolphins that died during a regional morbillivirus epizootic (Cruickshank et al., 1990; Inskeep et al., 1990). However, *T. gondii* has also been described in DMV-free dolphins (Mikaelian et al., 2000), suggesting that other factors may be implicated in the pathogenesis of this disease in cetaceans. The possibility of concurrent DMV infection was ruled out in this case by immunohistochemistry. However, some histopathologic findings such as lymphoid depletion and necrotizing stomatitis, compatible with opportunistic bacterial or viral infections (or both) (Jones et al., 1997), suggest that this animal was most likely immunosuppressed. Moreover, increased susceptibility to *T. gondii* has been reported in animals during pregnancy (Thouvenin et al., 1997). Despite insufficient studies in cetaceans, PCBs contamination is known to cause immunossuppression in other mammals (Vos and Luster, 1989), increasing the mortality from viral, bacterial, and protozoan diseases (Loose et al., 1978; Thomas and Hinsdill, 1978; Imanishi et al., 1980), and is known to be high in the Mediterranean Sea (Borrell et al., 1996). PCBs levels were not assessed in this dolphin, and their possible implication in this case is speculative.

This study reports transplacental infection in the early stages of pregnancy in a dolphin dying of disseminated toxoplasmosis. Among ceta-



FIGURES 1–7. *Toxoplasma gondii* and lesions in the brain of the mother dolphin (1–3) and in the fetal tissues (4–7). Bars in Figures 1–3, 6, 7 = 20 μm and in Figures 4, 5 = 100 μm. Figures 1, 5, and 7: immunohistochemical stain with anti–*T. gondii* antibodies, Figures 2, 3, 4, and 6: hematoxylin and eosin stain. (**1.**) Foci of gliosis with 3 tachyzoites. (**2.**) Foci of gliosis with many tachyzoites but only a few (arrows) are visible. (**3.**) Focal nonsuppurative inflammation with 5 tissue cysts (arrows) and 1 group of tachyzoites (arrow). (**4, 5.**) Focal necrosis and degenerating tachyzoites (all brown spots) in lung. (**6.**) Myocardium with focal necrosis (arrow) with many tachyzoites, but only a few are visible (arrowheads). (**7.**) Necrosis (arrow) and tachyzoites (arrowheads) in the heart.

ceans, transplacental *T. gondii* transmission has been observed in an Indo-Pacific bottlenose dolphin stillborn late-term fetus; however, no disease was observed in the dam (Jardine and Dubey, 2002). Most probably, this dam acquired a primary *T. gondii* infection rather than a relapse from reactivation of latent infection, with subsequent severe transplacental fetal infection, and developed fatal toxoplasmosis because of probable immunosuppression. The transplacental transmission in an early stage of gestation may be associated with the severe and disseminated lesions observed in the fetus. Thus, toxoplasmosis should be included in the differential diagnosis of reproductive failure in cetaceans.

The source of contamination in this dolphin is unknown, but it is likely that aquatic *T. gondii* infections are caused by an extension of the terrestrial life cycle. Sand and coastal waters can be contaminated

directly by oocysts shed in the feces of feral cats or indirectly by sewage effluents and serve as a source of infection for pelagic marine mammals. Moreover, aquatic birds or infected meat thrown into the sea may also be sources of infection for cetaceans. Fish and squid, the primary diet components of *G. griseus* (Carwardine, 1995), have not been identified as intermediate or transport hosts for *T. gondii*.

The prevalence of *T. gondii* infection is unknown in the Mediterranean cetacean populations. However, in the laboratory in Barcelona this is the fifth diagnosis of toxoplasmosis in dolphins, suggesting that this disease may be a frequent infection in this species, and that fatal cases may occur in immunosuppressed animals either by reactivation of latent infection or as a result of increased host susceptibility to primary infection. Serological studies would be necessary to assess this hypothesis.

The authors are grateful to Blanca Pérez and Pere Losada for their assistance with histotechnology. The authors would also like to thank Manuel García Hartmann from the Zoo of Duisburg and Jesús Ruberte from the Department of Embryology and Anatomy of the Veterinary School of Barcelona for critical opinion in fetal development, and to Dr. David Ferrer, Department of Parasitology, Veterinary School of Barcelona for critically reading the manuscript.

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